

## Research Note

Elimination of *Listeria monocytogenes* from Ready-to-Eat Turkey and Cheese Tortilla Wraps Using Ionizing Radiation<sup>†</sup>

CHRISTOPHER H. SOMMERS\* AND GLENN BOYD

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

MS 03-544: Received 25 November 2003/Accepted 9 April 2004

## ABSTRACT

*Listeria monocytogenes* is a common postprocess contaminant on ready-to-eat foods including premade ready-to-eat sandwiches. One popular type of sandwich product is the tortilla wrap, which contains sliced luncheon meats and cheeses rolled within a flour tortilla. This study determined the radiation resistance of *L. monocytogenes* surface inoculated onto two types of commercially available wheat flour tortillas, processed cheese slices, and deli turkey meat. The  $D_{10}$ -values for *L. monocytogenes* (the radiation dose required to inactivate 1 log of the pathogen) were 0.27 kGy when inoculated onto two flour tortilla types, 0.28 and 0.30 kGy when inoculated onto two types of sliced processed cheeses, and 0.58 and 0.65 kGy when inoculated onto two types of sliced deli turkey meat. When two types of tortilla wraps were assembled from the individual components and *L. monocytogenes* was inoculated into the interfaces between the individual components, the  $D_{10}$ -values were 0.27 to 0.37 kGy in the tortilla and cheese interfaces, 0.33 to 0.41 kGy in the cheese and turkey interfaces, and 0.25 to 0.33 kGy in the turkey and tortilla interfaces. The ability of ionizing radiation to reduce pathogen levels on the complex tortilla, cheese, and luncheon meat product was limited by the higher radiation resistance of *L. monocytogenes* when inoculated onto the ready-to-eat turkey-meat component.

*Listeria monocytogenes* is a frequent postprocess contaminant on ready-to-eat (RTE) food products including meats, cheeses, and sandwiches. The pathogen produces a high mortality rate among at-risk populations and is given zero tolerance in RTE foods by the U.S. Department of Agriculture Food Safety and Inspection Service (9–12, 20–22). In the last several years, over 100 million pounds of RTE food products, including meats, cheeses, and RTE sandwich products, contaminated with *L. monocytogenes* and other foodborne pathogens, have been recalled by the Food and Safety Inspection Service (19). Wallace et al. (21), in a survey of frankfurter packs obtained from several commercial production facilities, found that 1.6% of frankfurter packs were contaminated with *L. monocytogenes*. Gombas et al. (10) found 0.89% of luncheon meats and 0.17 to 1.42% of various cheeses to be contaminated with *L. monocytogenes*. Wilson (23) found that 15% of retail sandwiches were contaminated with *Listeria* spp.

Ionizing radiation can eliminate *L. monocytogenes* from RTE food products (6, 8, 14, 16, 17). Sommers and Thayer (17) determined that a radiation dose of 2.45 to 3.55 kGy was needed to reduce *L. monocytogenes* levels by 5 log CFU on RTE meats, while Foong et al. (8) found that a dose of 2.5 to 3.0 kGy was required to eliminate 5 log

CFU from a smaller subset of RTE meat products. Elimination of *L. monocytogenes* from cheese, like RTE meats, has been found to be formulation dependent, with the radiation dose needed to eliminate 1 log CFU from cheese varieties ranging from 0.41 to 1.38 kGy (4, 16, 18).

Research indicates an increasing trend in consumer purchase of RTE convenience-type foods (3). RTE sandwiches account for 32% of sales from vending machines and are a multibillion dollar annual business in the United States (1). These products are often manufactured in large processing facilities and distributed to thousands of vending machines and retail outlets on a daily basis over large metropolitan areas. One popular RTE sandwich-type food readily available for sale in both vending machines and convenience food outlets are tortilla wraps made with deli meats and cheese products. Little, if any, data are available on the radiation dose needed to eliminate *L. monocytogenes* from bread and Hispanic breads, such as tortillas. The purpose of this study was to (i) determine the radiation sensitivities on tortilla wraps, processed cheese, and sliced deli turkey meat individually and (ii) to examine the effect of component interaction on the radiation sensitivity of *L. monocytogenes* in assembled RTE tortilla, turkey, and cheese wraps.

## MATERIALS AND METHODS

**Food products.** Turkey, tortillas, and presliced processed and pasteurized American-style cheese were purchased from local vendors. Turkey deli meat was purchased in bulk and sliced to a

\* Author for correspondence. Tel: 215-836-3754; Fax: 215-233-6445; E-mail: csommers@arserrc.gov.

<sup>†</sup> Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

thickness of approximately 2 mm using an automatic meat slicer. The cheese was purchased as vacuum-packaged presliced bulk packs. Tortilla packs each contained 10 to 12 wheat flour-based wraps per package. In order to investigate more than a single turkey, cheese, or tortilla product, two types of tortilla, processed cheese, and turkey meat were used in the study and were designated by number (either 1 or 2) for that product.

Tortilla 1 consisted of enriched wheat flour (wheat flour, niacin, iron, thiamine mononitrate, riboflavin, folic acid), water, shortening (partially hydrogenated soybean and cottonseed oils with mono- and diglycerides added), salt, distilled monoglycerides, sodium bicarbonate, sodium aluminum phosphate, potassium sorbate and calcium propionate (to protect flavor), sugar, dough conditioners (sodium stearoyl lactylate, calcium sulfate, sodium sulfite), fumaric acid. Tortilla 2 consisted of enriched flour (wheat flour, niacin, reduced iron, thiamine mononitrate [B1], riboflavin [B2], folic acid), water, soybean oil, salt, potassium sorbate (used to preserve freshness), guar gum, fumaric acid, baking powder (sodium bicarbonate, sodium aluminum sulfate, cornstarch, calcium sulfate, monocalcium phosphate), sodium metabisulfite (dough conditioner).

Cheese 1 consisted of cultured milk and skim milk, water, cream, sodium citrate, salt, citric acid, sorbic acid (preservative), sodium phosphate, enzymes, lecithin. Cheese 2 consisted of cultured milk and skim milk, water, cream, sodium citrate, salt, sodium phosphate, sorbic acid (preservative), citric acid, artificial color, acetic acid, enzymes, and lecithin.

Turkey 1 consisted of white turkey meat, turkey broth, salt, modified food starch, sugar, carrageenan, sodium phosphate. Turkey 2 consisted of turkey breast meat, turkey broth, 2% or less of dextrose, modified food starch, salt, sodium erythorbate, sodium nitrite, and sodium phosphate.

**Bacterial strains.** Four *L. monocytogenes* strains (H7762 serotype 4b, H7764 serotype 4b, F4249 serotype 1/2a, and F4561 serotype 1/2a) were obtained from the Centers for Disease Control and Prevention (Atlanta, Ga.). The strains were propagated on tryptic soy agar (Difco, Becton Dickinson, Sparks, Md.) at 37°C and maintained at 0 to 4°C until used. Identity of *Listeria* spp. was confirmed by Gram stain followed by analysis with gram-positive identification cards using the Vitek Automicrobic System (bioMérieux Vitek, Inc., Hazelwood, Mo.).

**Inoculation.** Each *L. monocytogenes* strain was cultured independently in tryptic soy broth (Difco) in baffled, 500-ml Erlenmeyer culture flasks at 37°C (150 rpm) for 18 h. The cultures were then combined and the cocktail sedimented by centrifugation ( $1,725 \times g$  for 30 min). The *L. monocytogenes* cocktail was then concentrated by resuspension in 40 ml of Butterfield's phosphate buffer (Applied Research Institute, Newtown, Conn.). Turkey slices, cheeses slices, or tortilla quarters (approximately 60 cm<sup>2</sup>) were placed in no. 400 Stomacher bags and surface inoculated on a single side with 0.1 ml ( $10^8$  CFU) of *L. monocytogenes* cocktail. The inocula were then spread over the product surfaces using sterile, wet (Butterfield's phosphate buffer) cotton swabs.

Uninoculated wraps were assembled by single layering cheese slices and then turkey slices onto an intact tortilla wrap, which was then rolled to form the final product. Two types of wraps were assembled. Wrap 1 consisted of tortilla 1, cheese 1, and turkey 1, and wrap 2 consisted of tortilla 2, cheese 2, and turkey 2. The wraps were then cut into 3- to 4-cm-long sections, placed in no. 400 Stomacher bags, and inoculated with 0.1 ml ( $10^8$  CFU) of *L. monocytogenes* cocktail between the meat and cheese interface, the tortilla and cheese interface, or the tortilla and meat interface using a handheld pipette and 0.2-ml needle-

point pipet tip. All bags containing samples were sealed under aerobic conditions and maintained at 0 to 4°C for approximately 60 min until irradiation.

**Gamma irradiation.** A Lockheed Georgia Company (Marietta, Ga.) self-contained <sup>137</sup>Cs radiation source was used for all exposures. The dose rate (0.095 kGy/min) was verified according to methodology outlined by ASTM E 2116-00 (2) using dosimeters obtained from the National Institute of Standards and Technology. The temperature during irradiation was maintained at  $4.0 \pm 1.0^\circ\text{C}$  by the gas phase of a liquid nitrogen source that was introduced directly into the top of the sample chamber. The temperature was monitored using two thermocouples placed on the side of the sample bags. The absorbed dose was verified by use of 5-mm alanine pellet dosimeters that were attached to the sides of the sample bags, which were then measured using a Bruker EMS 104 EPR Analyzer (Billerica, Mass.). Recorded doses were typically  $\pm 5\%$  of the target doses, with a  $D_{\text{max}}/D_{\text{min}}$  ratio of  $\leq 1.1$  to 1.0 (17).

**Microbial analysis.** Following irradiation, the samples were assayed for CFUs by standard pour-plate procedures. One hundred milliliters of sterile Butterfield's phosphate buffer was added to a no. 400 Stomacher bag that contained a sample, and the sample was mixed by stomaching for 90 s. The samples were then serially diluted in Butterfield's phosphate buffer, using 10-fold dilutions, and 1 ml of diluted sample was pour plated using *Listeria*-specific Palcam Agar (Difco). Three 1-ml aliquots were plated per dilution. The plates were then incubated for approximately 48 h at 37°C prior to enumeration. The plates were scored for CFU using a calibrated AccuCount 1000 (AccuCount, Inc., Gainesville, Va.) colony counter.

**$D_{10}$ -values.**  $D_{10}$  is defined as the radiation dose required to achieve a 90% reduction in viable microorganism. The average CFU per gram of an irradiated sample ( $N$ ) was divided by the average CFU per gram of the untreated control ( $N_0$ ) to produce a survivor ratio ( $N/N_0$ ).  $D_{10}$ -value was determined by calculating the reciprocal of the slope provided by the log ( $N/N_0$ ) ratios versus irradiation dose (18).

**Statistical analysis.** Each experiment was conducted independently three times ( $n = 3$ ). Comparison of  $D_{10}$ -values was performed using analysis of covariance using Statistical Analysis Software Version 8 (SAS Institute, Cary, N.C.). Confidence intervals were determined using SigmaPlot 2000 (SPSS, Inc., Chicago, Ill.).

## RESULTS AND DISCUSSION

When *L. monocytogenes* was surface inoculated onto individual wrap components, i.e., tortillas, cheese, or deli turkey meat, the microorganism was more sensitive to ionizing radiation on tortillas or processed cheese slices as opposed to deli turkey meat. The  $D_{10}$ -values for *L. monocytogenes* on both types of tortilla wrap types were  $0.27 \pm 0.1$  kGy (Fig. 1). The  $D_{10}$ -values for *L. monocytogenes* on the two cheese types were  $0.28 \pm 0.1$  kGy for cheese 1 and  $0.30 \pm 0.2$  kGy for cheese 2, respectively (Fig. 1). It should be noted that radiation doses  $\geq 2.0$  kGy resulted in off-odor generation in the processed cheeses and were much more significant when nonprocessed cheeses such as provolone or Swiss cheese were irradiated (Sommers, unpublished observation). There was no statistically significant difference in  $D_{10}$ -value for *L. monocytogenes* inocu-

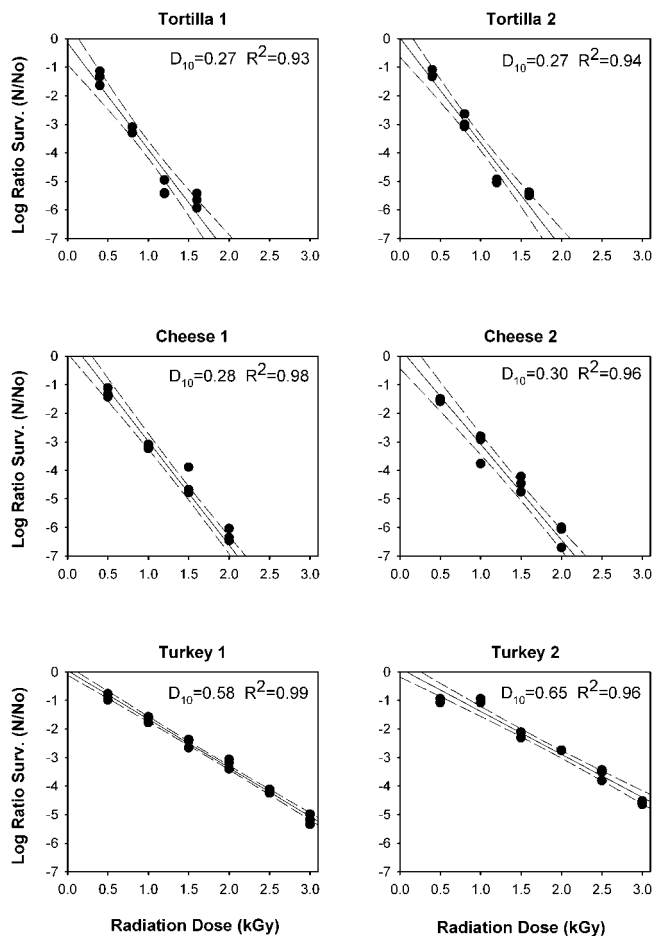


FIGURE 1. Radiation resistance of *Listeria monocytogenes* surface inoculated onto flour tortillas, processed cheese slices, and sliced deli turkey meat. Each experiment was conducted independently three times. Individual log reduction points are shown as closed circles, the linear regressions are shown as a solid lines, and the 95% confidence intervals are shown as dashed lines.

lated onto either tortillas or processed cheese (analysis of variance) ( $n = 3$ ,  $\alpha = 0.05$ ). For both product types, cheese and tortillas, a  $\geq 5$ -log reduction was obtained at a radiation dose of 1.6 kGy.

In contrast with  $D_{10}$ -values on tortillas and cheese, the  $D_{10}$ -values for *L. monocytogenes* inoculated onto deli turkey meat were much higher,  $0.58 \pm 0.2$  kGy for turkey 1 and  $0.65 \text{ kGy} \pm 0.3$  kGy for turkey 2 (Fig. 1). At a radiation dose of 2.0 kGy, the radiation dose at which off odors were noticed with the cheeses, a 3.1- to 3.8-log reduction of *L. monocytogenes* would be obtained. The  $D_{10}$ -values were statistically significantly higher than those obtained for *L. monocytogenes* on turkey as opposed to the cheese and tortilla types as determined by analysis of variance ( $n = 3$ ,  $\alpha = 0.05$ ).

When *L. monocytogenes* was inoculated between the component layers of assembled wrap products, the microorganism retained radiation sensitivity intermediate or equivalent to that of the two individual component types. *L. monocytogenes*  $D_{10}$ -values were 0.37 and 0.27 kGy when inoculated between cheese and tortilla for wrap 1 and wrap 2, respectively (Fig. 2).  $D_{10}$ -values were 0.33 and 0.25

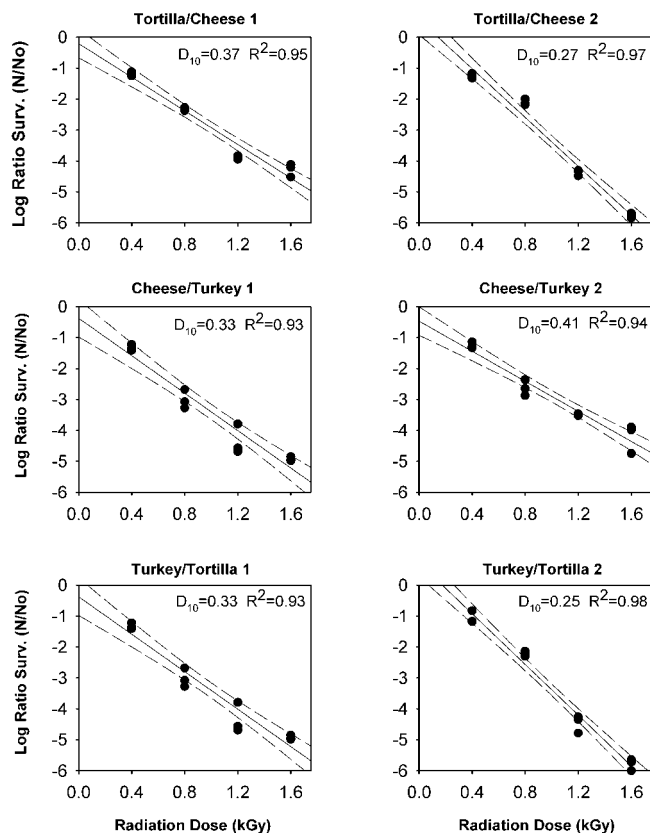


FIGURE 2. Radiation resistance of *Listeria monocytogenes* surface inoculated into interfaces between flour tortillas, processed cheese slices, and sliced deli turkey meat in assembled turkey and cheese tortilla wraps. Each experiment was conducted independently three times. Individual log reduction points are shown as closed circles, the linear regressions are shown as a solid line, and the 95% confidence intervals are shown as dashed lines.

kGy when inoculated between turkey and tortilla for wrap 1 and wrap 2, respectively (Fig. 2). *L. monocytogenes*  $D_{10}$ -values were 0.33 and 0.41 kGy when inoculated between the cheese and turkey interfaces for wrap 1 and wrap 2, respectively (Fig. 2). All of the  $D_{10}$ -values for *L. monocytogenes* obtained from inoculation into the component interfaces were significantly less than the  $D_{10}$ -values for *L. monocytogenes* inoculated onto deli turkey meat (analysis of variance) ( $n = 3$ ,  $\alpha = 0.05$ ). Log reductions for *L. monocytogenes*, inoculated between component layers, at a radiation dose of 2.0 kGy, would range between 4.5 and 8 log.

Data obtained from irradiation of these complex food products are in agreement with previous studies that indicate that product formulation affects the radiation resistance of *L. monocytogenes*. Sommers and Thayer (17) found that the  $D_{10}$ -values of *L. monocytogenes* inoculated onto several types of commercially available frankfurters ranged from 0.49 to 0.71 kGy. Foong et al. (8) found that the radiation dose needed to eliminate 5 log of *L. monocytogenes* from RTE meats ranged from 2.5 to 3.0 kGy. Ennhar et al. (7) determined the  $D_{10}$ -values of *L. monocytogenes* inoculated onto soft and red-smear cheeses to be 0.49 and 0.41 kGy respectively, while Tsiotsias et al. (18) found the  $D_{10}$  of *L. monocytogenes* inoculated onto Anthotyros cheese to be



1.38 kGy. Bougle and Stahl (4) obtained a  $D_{10}$ -value of 0.50 kGy for *L. monocytogenes* inoculated onto Camembert cheese. Little data are available pertaining to the radiation resistance of *L. monocytogenes* inoculated onto different types of bread products.

Based on the radiation resistance on individual components for *L. monocytogenes* and when the pathogen is inoculated between product layers, a radiation dose of 2.0 kGy is sufficient to eliminate  $\geq 5$  log of the pathogen from tortillas, cheese, and the interfaces between them. Three to 4 log CFU/g of the microorganism would be removed from the deli meat. Previous work (16) has indicated that a radiation dose of 1.5 kGy represses the postirradiation growth of *L. monocytogenes* inoculated onto RTE meats for 1 to 2 weeks and that postirradiation growth of the microorganism is completely inhibited when additives such as sodium diacetate and potassium lactate mixtures are included in RTE meat-product formulations (16). Given the short shelf life of such products,  $\leq 1$  week, and based on the data obtained in *L. monocytogenes* detection studies (5, 10, 23), a radiation dose of 2.0 kGy would eliminate the bacterium from 99.9% of RTE products and be unable to proliferate in the remainder. If additional protection were required, use of deli meats that contain additives such as sodium diacetate and potassium lactate mixtures for inclusion in the assembled RTE wrap products would ensure a minimum 3-log reduction without the possibility of *L. monocytogenes* regrowth in tortilla wrap-type products regardless of shelf life. Other approaches might include the use of modified atmospheres packaging in combination with irradiation to inhibit microbial growth. Future work should include quality and organoleptic studies of an irradiated product.

While the vast majority of luncheon meats and bagged salad vegetables contaminated with *L. monocytogenes* contain relatively low levels ( $\leq 10^2$  CFU/g) of the microorganism, it is not unusual that products subjected to temperature abuse ( $10^\circ\text{C}$ ) may contain  $\geq 10^4$  CFU/g of *L. monocytogenes* (10, 13, 23). Wilson (23) found that 15% of pre-packed retail sandwiches contained *Listeria* spp. and 0.3% of the contaminated sandwiches contained  $>10^3$  CFU/g of *L. monocytogenes*. In addition, Gombas et al. (10) noted that retailers that prepared and packaged RTE foods had *L. monocytogenes* detection rates in their products that ranged from 2.7 to 6.3%, much higher than those detected by products obtained directly from manufacturers (0.4 to 1.4%). Complex multicomponent products assembled for retail sale could therefore benefit from a postassembly and postpackaging pathogen-reduction treatment such as irradiation, which protects the public from foodborne illness outbreaks.

## ACKNOWLEDGMENTS

The authors thank Kym Snipes and Laren Melenski for technical assistance.

## REFERENCES

- Anonymous. 2001. Hot stuff. Food service sampler. From Rich Products. Spring TCP Publishing, Buffalo, N.Y.
- Anonymous. 2002. Practice for dosimetry for a self-contained dry storage gamma irradiator, p. 241–253. In Standards for radiation processing. ASTM International, Conshohocken, Pa.
- Anonymous. 2003. It's whats for dinner. *Prepared Foods* 172(04): 24.
- Bougle, D. L., and V. Stahl. 1994. Survival of *Listeria monocytogenes* after irradiation treatment of camembert cheeses made from raw milk. *J. Food Prot.* 57:811–813.
- Chen, Y., W. H. Ross, V. N. Scott, and D. E. Gombas. 2003. *Listeria monocytogenes*: low levels equal low risk. *J. Food Prot.* 66:570–577.
- Clardy, S., D. M. Foley, F. Caporaso, M. L. Calicchia, and A. Prakash. 2002. Effect of gamma irradiation on *Listeria monocytogenes* in frozen, artificially contaminated sandwiches. *J. Food Prot.* 65: 1740–1744.
- Ennhar, S., F. Kuntz, A. Strasser, M. Bergaentzle, C. Hasselman, and V. Stahl. 1994. Elimination of *Listeria monocytogenes* in soft and red smear cheeses by irradiation with low energy electrons. *Int. J. Food Sci. Technol.* 29:395–403.
- Foong, S. C., G. C. Gonzalez, and J. S. Dickson. 2002. Marginal safety of irradiation dosage for reduction and post-irradiation survival of *Listeria monocytogenes* in ready-to-eat (RTE) meats. Abstr. P043. Int. Assoc. Food Prot. Annu. Meet., San Diego, Calif.
- Gerba, C. P., J. B. Rose, and C. N. Haas. 1996. Sensitive populations: who is at risk. *Int. J. Food Microbiol.* 30:113–123.
- Gombas, D. E., Y. Chen, R. S. Clavero, and V. N. Scott. 2003. Survey of *Listeria monocytogenes* in ready-to-eat-foods. *J. Food Prot.* 66:559–569.
- Levine, P. B., B. Rose, S. Green, G. Ransom, and W. Hill. 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States. *J. Food Prot.* 64:1188–1193.
- Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.
- Odumeru, J. A., S. J. Mitchell, J. A. Alves, J. A. Lynch, A. J. Yee, S. L. Wang, S. Styliadis, and J. M. Farber. 1997. Assessment of the microbiological quality of ready-to-use vegetables for health care food services. *J. Food Prot.* 60:954–960.
- Schoeller, N. P., S. C. Ingham, and B. H. Ingham. 2002. Assessment of the potential for *Listeria monocytogenes* survival and growth during alfalfa sprout production and use of ionizing radiation as a potential intervention treatment. *J. Food Prot.* 65:1259–1266.
- Smith, J. L. 1998. Foodborne illness in the elderly. *J. Food Prot.* 61:1229–1239.
- Sommers, C. H., X. Fan, B. A. Niemira, and K. Sokorai. 2003. Radiation (gamma) resistance and post-irradiation growth of *Listeria monocytogenes* suspended in beef bologna that contained sodium diacetate and potassium lactate. *J. Food Prot.* 66:2051–2056.
- Sommers, C. H., and D. W. Thayer. 2000. Survival of surface-inoculated *Listeria monocytogenes* on commercially available frankfurters following gamma irradiation. *J. Food Safety* 20:127–137.
- Tsiotsias, A., I. Savvaidis, A. Vassila, M. Kontominas, and P. Kotzekidou. 2002. Control of *Listeria monocytogenes* by low-dose irradiation in combination with refrigeration in the soft whey cheese anthotyros. *Food Microbiol.* 19:117–126.
- U.S. Department of Agriculture. 2003. Meat and poultry product recalls: news releases and information for consumers. USDA, Food Safety Inspection Service, Washington, D.C. Available at: <http://www.fsis.usda.gov/OA/recalls/rec.all.htm>. Accessed 25 November 2003.
- U.S. Department of Health and Human Services and United States Department of Agriculture. 2001. Draft assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Washington, D.C. Available at: <http://www.foodsafety.gov/~dms/lmrisk.html>. Accessed 25 November 2003.
- Wallace, F. M., J. E. Call, A. C. S. Porto, G. J. Cocoma, The ERRC Special Project Team, and J. B. Luchansky. 2003. Recovery rate of *Listeria monocytogenes* from commercially prepared frankfurters during extended refrigerated storage. *J. Food Prot.* 66:584–591.
- Wilson, I. G. 1995. Occurrences of *Listeria* species in ready-to-eat foods. *Epidemiol. Infect.* 115:519–526.
- Wilson, I. G. 1996. Occurrence of *Listeria monocytogenes* in pre-packed retail sandwiches. *Epidemiol. Infect.* 117(1):89–93.